AppliedPhotophysics

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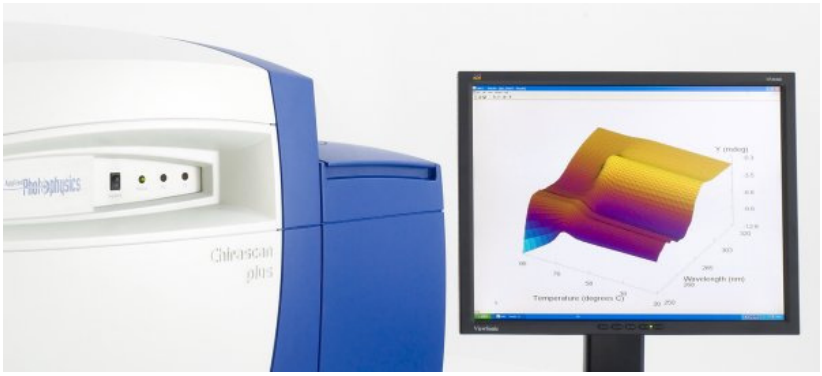
Spectroscopy Article
"Structure and Thermodynamics of a Monoclonal Antibody Biotherapeutic in Different Formulations"

Protein stability, formulation testing and quality control



A stable, correctly folded protein is an absolute requirement for a successful biotherapeutic, and the stability of a desired conformation is determined by suitable formulation.

The stability of a protein therapeutic is conventionally monitored by calorimetric techniques, particularly differential scanning calorimetry (DSC), yielding thermodynamic parameters such as the enthalpy and the mid-point of a conformational transition. The thermodynamic parameters are indicative of the relative stabilities of proteins in different conditions but calorimetric techniques cannot tell you how the conformation changes, whether or not a conformational change leads to aggregation, or if the protein is in its desired conformation prior to heating.

Knowledge of secondary structure will confirm whether or not the protein is correctly folded initially and observing the secondary structure as a function of temperature will tell you how its conformation changes on heating. However, the only common method to determine protein secondary structure in solution is circular dichroism (CD) and monitoring CD as a function of temperature can be a time-consuming business.

Using the technique of dynamic multimode spectroscopy (DMS) it is possible to use multiple spectroscopic probes to monitor changes in secondary structure as a function of temperature and to determine the thermodynamics of unfolding. Chirascan-plus DMS combines the benefits of spectroscopic and calorimetric measurements into a single, rapid, information-rich measurement that generates results from a complete experiment in about an hour.

Experiments of this type are used to prove that a protein is initially correctly folded and hence biologically active. It is also possible to distinguish between unfolding and aggregation steps which are of relevance to stability and potential immunogenicity. The Tm and enthalpy values can be determined as quantitative indicators of stability making Chirascan-plus a valuable orthogonal technique in the biophysics laboratory.

The technique has been applied in the Pharmaceutical industry to inform about 'safe' temperatures for accelerated stability studies and to screen for better formulations. Measurements taken in the near UV can be used to demonstrate more subtle changes in tertiary structure. Often these subtle changes occur at lower temperature and if they are irreversible then these conditions need to be avoided. Also, it may be known from protein engineering work that a key amino acid, for example tryptophan, maybe necessary for activity. In such cases formulations that induced changes in the tryptophan part of the spectra would be avoided.

The use of Chirascan™-plus Dynamic Multi-mode Spectroscopy (DMS) has recently been used for optimizing biotherapeutic formulation of a monoclonal antibody under different pH conditions. In this study, DMS is applied to the denaturation of a monoclonal antibody under different pH conditions to show the potential of the technology in biotherapeutic development.

Below are links to some application notes which describe DMS in more detail and demonstrate DMS in action.

A new approach for optimizing biotherapeutic formulation using Chirascan Dynamic Multi-mode Spectroscopy (DMS)

In this study, DMS is applied to the denaturation of a monoclonal antibody under different pH conditions to show the potential of the technology in biotherapeutic development.

Download the full Application Note here.

The thermal denaturation of an oligonucleotide: a study to validate dynamic multimode spectroscopy

In this study, an oligonucleotide is used to compare results from DMS and DSC and to demonstrate that the technique is reliable, reproducible and accurate.

Download the full Application Note here.

Testing the stability of antibody biotherapeutics in different formulations using dynamic multi-mode spectroscopy (DMS)

Another powerful application for Chirascan CD spectroscopy is to compare different macromolecules, or the same molecule under different conditions, and determine if they have a similar structure. This can be used to ascertain if a newly purified protein is correctly folded, to determine if a mutant protein has folded correctly in comparison to the wild-type, or for the analysis of biopharmaceutical products to determine that the product is still in a correctly folded active conformation.

This study describes an investigation into the stability of a monoclonal antibody biotherapeutic in two different formulation buffers (acetate and lactate). The technique used was dynamic multi-mode spectroscopy (DMS) and the work was carried out on a Chirascan-plus CD spectrometer.

Download the full Application Note here.

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